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PORPHYRIN INHIBITION OF GLYOXALASE I

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The use of porphyrins and light is of corrent interest in cancer studies both therapeutically and diagnostically (1). The ability of certain porphyrins to localise in neoplastic tissue has been exploited both for fluorescence detection (2) and tissue destruction on photoirradiation (3). The explanation of the latter has been suggested to be light-promoted singlet oxygen and free radical formation (3), but in reality neither the localisation nor tissue destructive properties of porphyrins are well understood. There in some indication in the literature that singlet oxygen/free radical processes may not be the only mechanism for tumour destruction. Thus, Yamamato has reported (4) that the binding of phytochlorin sodium to cancer cells in vitro under the influence of visible light was enhanced by methylglyoval. The methylglyoxal also lowered the level of phytochlorin sodium required for cytoplasmic swelling and loss of tumour-forming ability by almost three-fold. Yamamoto (4) suggested that this implied synergism between the a-ketoaldehyde and the porphyrin in cancer cell division. He also suggested that this might implicate the glyoxalase system in mitosis. In view of such proposals we have studied a number of porphyrins and found them to inhibit glyoxalase I from a number of sources.

Materials and methods: Yeast glyoxalase I (Grade IV), haemin and assay substrates were purchased from Sigma Chemical Co. (St. Louis, Mo.). Rat liver (5) and human erythroyte (6) glyoxalase I were isolated as previously descrbed. Glyoxalase I was assayed by allowing glutathione and methylglyoxal to equilibrate (5 min) at 25°C in pH 6.60 phosphate (0.05 m) butter,

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in the presence or absence of inhibitor as appropriate. The initial velocity was recorded spectrophotmetrically at 240 nm on addition of enzyme (7). Substrate concentrations were calculated using a

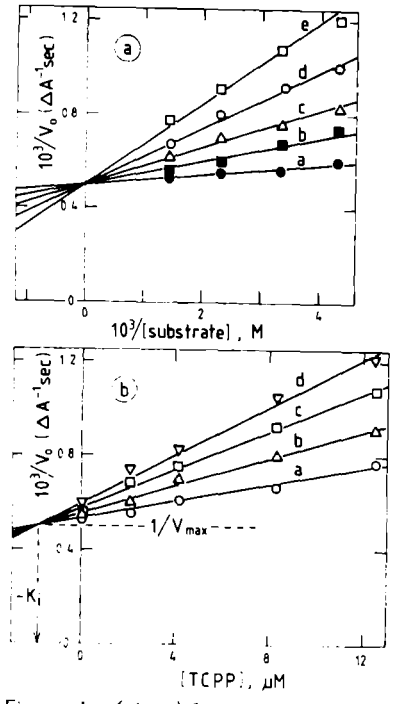

Figure 1a (above):Lineweaver-Burk plot ($1/V_0$ versus $1/S_0$) at various inhibitor concentrations for inhibition of rat liver glyoxalase I by meso- *tetra-(4-carboxyphenyl)-porphine (TCPP) at pH 6.60, 25 °C. Points are experimental: lines were obtained by linear least squares regression analysis of the data assuming linear competitive inhibition with $1/V_{max}$ = 0.503 × $10^3 \Delta A^{-1}$sec: concentrations of TCPP used were: (a) 0, (b) 2.08 μM (c) 4.16 μM, (d) 8.3 μM, (e) 12.5 μM). Figure 1b (below): Dixon plot of $1/V_0$ versus [TCPP] at various concentrations of hemimercaptal substrate for inhibition of rat liver glyoxalase I at pH 6.6, 25 °C. Initial concentrations of hemimercaptal were: (a) 0.700 mM; (b) 0.433 mM; (c) 0.300 mM; (d) 0.233 mM Points are experimental: lines are theoretical, by linear least squares regression analysis of the data, assuming K_i = 1.86 μM and linear competitive inhibition, with $1/V_{max}$ equal to 50.3 ΔA^{-1}sec.*

dissociation constant of 3.1 mM for the hemithiolacetale adduct (8). Mesotetra-(4-carboxyphenyl)-porphine tetrasodium salt (TCPP) and mesotetra-(4-sulphonatophenyl)-porphine tetrasodium salt ($TPPS_4$) were purchased from Strem Chemical Co. (Newburyport, Ma., USA).

Results: The inhibition of rat liver glyoxalase I by TCPP at pH 6.60 is shown in the figure. By both Lineweaver-Burk ($1/V_0$ versus $1/[So]$) and Dixon criteria ($1/V_0$ versus $[I_0]$) the inhibition is linear and competitive. Similary, for yeast enzyme at this pH linear competitive inhibition kinetics were observed for TCPP, $TPPS_4$ and haemin. Inhibition constants are recorded in the table. Glyoxalase I from human eryhrocytes was also strongly inhibited by TCPP, and less strongly by haemin and $TPPS_4$. However, a complex scheme of inhibition was followed by all three porphyrins with glyoxalase I from this species. Values of I_{50} (inhibitor concentratoins required to give 50% inhibition) were dependent on the substrate contration for some pH values and some inhibitors and are gven in the table. The inhibition of yeast glyoxalase I by TCPP and $TPPS_4$, was pH-dependent; for both compounds Ki values obeyed the Henderson-Hasselbach equation, showing that binding was controlled by ionisation of a group of pK_{app} 6.5 and 7.1 respectively. Inhibition was stronger at lower pH for the yeast enzyme (see table). In view of the more complex inhibition pattern shown with the human erythrocyte enzyme detailed pH dependence of the inhibition was not undertaken. However, I_{50} values for TCPP with this enzyme were measured at a number of pH values (at a substrate concentration of 1.15×10^{-4}M) and were as follows (pH in parenthesis: 5.85 μm (pH 6.60); 2.32 μM (pH 6.00; 2.14 μM (pH 5.50). Thus, inhibition by TCPP under these conditions also appears stronger at lower pH for human erythrocyte glyoxalase I.

Discussion: It is clear from the figure and table that glyoxalase I from a number of species is inhibited by porphyrns of various structures. The inhibition is straightforward (linear and competitive) for the yeast enzyme and the rat enzyme (TCPP) but more complex for that from human erythrocytes, indicating species differences. Even with human enzyme, relatively low 150 values were observed at pH 6.60; for the rat liver enzyme the Ki value for TCPP was 2 μM at pH 6.60.

The cellular level of methylglyoxal has not been reported to our knowledge but is likely to be very low. Thus, the substrate concentration for glyoxalase I will be low, a condition which emphasises porphyrin inhibition of the human erythrocyte enzyme under some conditions (see table). Such data support the view of Yamamoto (4) that the synergism he observed on co-irradiating cancer cells in the presence of phytochlorin sodium/methylglyoxal mixtures could be caused by an effect on the glyoxalase system.

Inhibition of glyoxalase I from various sources by porphyrins at pH 6.60, 25 °C, except where noted (means ± SD); the level of dimethylsulphoxide used for porphyrin solubilisation in the assay was ≤ 0.125%

	Glyoxalase I from			
			Human erythrocytes	
Porphyrin	Yeast Ki (μM)	Rat liver Ki (μM)	I_{50} (μM)	at $[S_0]$ (M)
TCPP	13.5 ± 1.5[a]	1.86 ± 0.59[e]	6.2[d]	2.30×10^{-4}
			10.5	4.20×10^{-4}
$TPPS_4$	61.2 ± 7.3[b]		21	2.30×10^{-4}
			> 40	4.20×10^{-4}
Haemin	66.5 ± 0.8[c]		> 100	All values used

The Ki values were as follows:- a) at pH 6.07, 2.60 (± 0.20) × 10^{-6}M; b) at pH 5.40, 7.90 (± 0.01) × 10^{-6}M; c) at pH 5.33, 9.1 (± 0.5 × 10^{-6}M and at pH 6.00, 2.02 (± 0.42) × 10^{-5}M. d) At pH 6.0, the I_{50} value was independent of substrate concentration (S_0 = 1.68–2.30 × 10^{-4}M). e) From a Dixon analysis; the K_i from the same data by replotting the slopes of the double reciprocal plot (Lineweaver-Burk) versus [I] was 2.67 (± 0.85) × 10^{-6}M.

The inhibition by TCPP, $TPPS_4$ and haemin is much stronger at lower pH for the yeast enzyme and it is possible that extrapolation to glyoxalase I from other tissues can be made in view of the I_{50} data for the human erythrocyte enzyme at lower pH. As cancer cells, especially after glucose treatment, have lower pH values that normal tissue (9), glyoxalase I inhibition by porphyrins may be stronger than in normal cells. This type of interaction may explain the anticancer synergism (4) between porphyrins and methylglyoxal under photoirradiation.

It is not clear whether the glyoxalase-binding activity of porphyrins contributes to their selective uptake by cancerous tissue, as several other cellular components can bind porphyrins tightly, e.g., glutathions S-transferase (10).

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